

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/564, C07K 1/22, 16/18, A61K 31/70, 39/395		A1	(11) International Publication Number: WO 96/27131
			(43) International Publication Date: 6 September 1996 (06.09.96)
(21) International Application Number: PCT/FI96/00117			(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 29 February 1996 (29.02.96)			
(30) Priority Data: 950962 1 March 1995 (01.03.95) FI			
(71)(72) Applicant and Inventor: SALONEN, Eeva-Marjatta [FI/FI]; Tunturikatu 15 B 46, FIN-00100 Helsinki (FI).			
(74) Agent: BORENIUS & CO. OY AB; Arkadiankatu 21 A, FIN-00100 Helsinki (FI).			
Published <i>With international search report.</i>			
(54) Title: ANTI DNA ANTIBODY DETECTION INVOLVING TELOMERIC DNA SEQUENCE RECOGNITION AND BINDING			
(57) Abstract <p>The invention relates to a diagnostic method for detection of anti-DNA antibodies in patients with lupus erythematosus, particularly SLE, test kit, solid phase means and a therapeutic method and a drug. All these applications are characterised by the use of telomeric DNA sequences able to bind specifically to anti-DNA-antibodies. Preferred antibody-binding telomeric sequences comprise 5'-TTAGGG-3', 5'-CCCTAA-3', repeats thereof or double stranded vertebrate telomere. Solid phase removal of anti-DNA antibodies with solid phase bound telomeric sequences and use of the telomeric sequences as a drug for treatment of patients with autoimmune disorders are revealed.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

Anti DNA antibody detection involving telomeric DNA
sequence recognition and binding

FIELD OF INVENTION

The present invention relates to a method for detecting anti-DNA-antibodies in samples obtained from eukaryotic organisms, especially in vertebrate body fluids, and more particularly to a diagnostic method for detecting anti-DNA-antibodies in body fluids from human beings and domestic animals. The invention also relates to test kits for performing the said method and to drugs and therapy for patients suffering from diseases involving the presence of anti-DNA-antibodies, specifically autoimmune disorders such as Lupus Erythematosus and Rheumatoid Arthritis.

BACKGROUND OF THE INVENTION

Lupus erythematosus is an autoimmune disorder (antibodies are produced against self antigens), in which the body's immune system, for unknown reasons, attacks the connective tissue as though it were foreign, causing inflammation. Of the multitude of autoreactive antibodies that spontaneously arise during the disease, high levels of circulating autoantibodies to DNA are the best evidence of the pathogenesis.

In Systemic Lupus Erythematosus (SLE) there is almost invariable presence in the blood of antibodies directed against one or more components of cell nuclei. Certain manifestations in SLE seem to be associated with the presence of different antinuclear antibodies and genetic markers, which have suggested that SLE may be a family of diseases [Mills, J.A., Medical Progress 33, 1871-1879 (1994)].

The more common type of lupus erythematosus, Discoid Lupus Erythematosus (DLE), affects exposed areas of the skin. The more serious and fatal form, Systemic Lupus Erythematosus (SLE), affects many systems of the body, including the joints and the kidneys.

Animal models have confirmed that organ damage and premature death occurs only following the skewing of the B cell repertoire towards autoreactivity

[Klinman, D.M. J.Clin. Invest. 86, 1249-1254 (1990). Klinman, D.M. et al., J.Immunol. 144, 506-511 (1990)]. Lupus nephritis, especially diffuse proliferative glomerulonephritis, has been known to be associated with circulating antibodies to double-stranded (native) DNA [Casals, S. et al., Arthritis Rheum. 7, 379-390 (1964); Tan E.M. et al., J.Clin. Invest. 82, 1288-1294 (1966)]. The detection of antinuclear antibodies is a sensitive screening test for SLE. Antinuclear antibodies occur in more than 95 % of patients [Hochberg, M.C. Rheum. Dis. Clin. North Am. 16, 617-39 (1990)].

The most common antibody in patients with SLE is directed against nucleosomal DNA-histone complexes, and it yields a homogeneous staining pattern on the immunofluorescence test for antinuclear antibodies [Mohan, C. et al., J. Exp. Med. (1993) 177, 1367-81]. Antinuclear antibodies are also seen in most of the other rheumatic diseases, [Tan, E.M., Adv. Immunol. (1989) 44, 93-151] and are produced transiently in viral infections and are present, usually in low titers, in about 2 percent of the normal population.

Antibodies to native or double-stranded DNA and to Sm, a ribonuclear protein antigen, are more specific than other antinuclear antibodies for the diagnosis of SLE. [Mills, J.A., Medical Progress 33, 1871-1879 (1994)]. It is more common to have nephritis in patients with antinative DNA, the titer of this antibody being a useful measure of disease activity [Swaak, A.J.G. et al., Ann. Rheum. Dis. 1986, 45: 359-66; ter Borg, E.J. et al., Arthritis Rheum. 1990: 33, 634-43].

The 10-year average survival rate from diagnosis for patients with SLE observed over the past decade approaches 90 percent [Pistiner et al., Semin. Arthritis Rheum. 1991: 21, 55-64; Ginzler et al., Rheum. Dis. Clin. North Am. 1988: 14, 67-78].

During 1993-1994 the present inventor was working at the Scripps Research Institute (La Jolla, CA, USA) in a project involving a human antibody gene library from a Systemic Lupus Erythematosus (SLE) donor. The library was panned against human placental DNA (a commercial product), and selected monoclonal antibodies were tested for human antibody recognition of DNA. The results of the project are disclosed in Barbas, S.M., Ditzel, H.J., Salonen, E.-M., Yang, W.-P., Silverman, G.J. and D.R. Burton, Human autoantibody recognition of DNA, Proc. Natl. Acad. Sci. 1995: 92, 2529-2533. The object

was to discover a specific sequence of DNA that would be recognized by a human SLE autoantibody.

A haploid set of human chromosomes (haploid human genome) is composed of the estimated 3 billion (3×10^9) bp of DNA. The probability of finding, experimentally, a short sequence that is recognized by autoantibodies is small. Thus, despite a vast amount of work, a functionally correct sequence has not been found.

SUMMARY OF THE INVENTION

Subsequently, the present inventor in her studies in another context has unexpectedly found that synthesized telomeric sequences, i.e. sequences that are found at the terminal ends of the chromosomes bind the autoantibodies in question and that they can be used to detect and to inhibit said anti-DNA-antibodies.

It is therefore an object of the present invention to provide a method for detecting anti-DNA-antibodies in eukaryotic samples.

More specifically, it is an object of the invention to provide a method for detecting anti-DNA-antibodies in body fluids such as serum or cerebrospinal fluid from vertebrates, especially human beings.

It is a further object of the invention to provide a diagnostic method for the detection of Lupus Erythematosus, Rheumatoid Arthritis, Scleroderma and other autoimmune diseases, especially Systemic Lupus Erythematosus in vertebrates, by detecting an elevated level of anti-DNA-antibodies in a body fluid sample of said vertebrate with the aid of telomeric sequences specific to said mammal and/or with the aid of antibodies or fragments of antibodies specific to said telomeric sequence.

In the following specification and claims the term "telomeric sequence" used generally in context with the invention should be understood as meaning any of the telomeric sequences of DNA selected from the group consisting of a single-stranded vertebrate telomeric sequence, a complementary strand thereto, a double stranded vertebrate telomere, and a part or a repeat or a combination of any of the foregoing, which is capable of binding said

anti-DNA-antibodies.

The term antibody specific to said telomeric sequence should be understood as meaning any antibody which specifically binds to said telomeric sequence and hence is capable of competition with said anti-DNA-antibody. Said specific antibody may be of any Ig class, but it is preferably an antibody of the IgG, IgM or IgA class. Special benefits of the invention are obtained by the use of fragments of the specific antibody, especially Fabs or F(ab')₂s. Said fragments may be derived from naturally occurring anti-DNA-antibodies or they may preferably be produced by recombinant DNA techniques.

It is also an object of the invention to provide a test kit for detecting anti-DNA-antibodies, said test kit preferably including immobilized telomeric sequences capable of binding to anti-DNA-antibodies. The test kit preferably includes a label indicating the binding of anti-DNA-antibody to telomeric sequence.

An object of the invention is to provide both quantitative and qualitative test kits for the detection of anti-DNA-antibodies.

It is also an object of the invention to provide a means for the removal of anti-DNA-antibodies from a liquid sample, especially from a body fluid of a vertebrate, such as the serum of a human being by selectively binding said anti-DNA-antibodies to telomeric sequences outside the body of said vertebrate. Said means preferably comprises a column or matrix including immobilized telomeric sequences capable of binding anti-DNA-antibodies.

It is a further object of the invention to provide a drug capable of inhibiting the binding or reducing the activity of anti-DNA-antibodies in patients suffering from autoimmune disorders, which drug contains an amount of telomeric sequences or fragments of antibodies thereto effective in inhibiting the telomeric sequence binding or neutralizing the destructive action of the patient's specific anti-DNA-antibodies.

It is a further object of the invention to provide a method useful in the treatment of autoimmune disorders by reducing or inhibiting the activity of anti-DNA-antibodies in a vertebrate system by administering to said vertebrate an effective amount of a vertebrate telomeric sequence or an agent containing

a vertebrate telomeric sequence or a functional part thereof, or a fragment of an antibody thereto, which results in a significant inhibition or neutralization of the action of the anti-DNA-antibodies in said vertebrate.

Telomeres, terminal DNA-protein complexes of chromosomes, are essential in the protection, positioning and for the stability of chromosomes [E.H. Blackburn and J.W. Szostak, Annu. Rev. Biochem. 53, 163 (1984); V.A. Zakian, Annu. Rev. Genet. 23, 579 (1989)]. They also are required for the complete replication of the chromosomal terminus during each cell cycle. All known eukaryotic telomeres consist of hundreds to thousands of simple, repeated tandem sequences of DNA and associated proteins. A functional human telomere is defined as having a repeat of the sequence 5'-TTAGGG-3' [E.H. Blackburn, Nature 350, 569 (1991); R.K. Moyzis et al., Proc. Natl. Acad. Sci. U.S.A. 85, 6622 (1988); J. Meyne et al. Proc. Natl. Acad. Sci. U.S.A. 86, 7049 (1989)]. All vertebrates have the same telomeric sequence (TTAGGG)_n [Moyzis, R.K., Buckingham, J. M., Cram, L.S., Dani, M., Deaven, L.L., Jones, M. D., Meyne, J., Ratliff, R. L. & Wu, J.-R. (1988) Proc. Natl. Acad. Sci. USA 85, 6622-6626, J. Meyne, R. L. Ratliff, R. K. Moyzis, Proc. Natl. Acad. Sci. U.S.A 86, 7049 (1989)]. A general structure of repeated sequences of G- and C-rich complementary strands of the eukaryotic telomeres is (T or A)_m(G)_n [Blackburn, E.H. and Szostak, J.W. Annu. Rev. Biochem. 53, 163-194 (1984); Weiner, A.M. Cell 52, 155-157 (1988)].

Telomeres can be visualized at the tips of the chromosomes using a fluorescence microscope. When labeled (GGGTTA)₇-(TAACCC)₇ oligomers were hybridized to metaphase chromosomes using a biotin-avidin detection method the distinct, speckled fluorescent signal was distinctly seen at the chromosomal ends [Meyne, J.M. et al., Proc. Natl. Acad. Sci. 86, 7049-7053 (1989)]. Although telomeric sequences are highly conserved in evolution, the correct sequence of the telomeric repeat is required for telomere function, since, for example, addition of telomeric DNA harboring a mutated telomeric sequence to the ends of the endogenous *Tetrahymena* telomeres (having a sequence repeat 5'-TTGGGG-3') leads to telomere length instability and death [Yu et al., Nature (London) 344, 126-132 (1990); Harley, C.B. et al., Nature (London) 345, 458-460 (1990)].

The structure and function of telomeres has been described by E.H. Blackburn, Nature, 350, 569-573 (1991). Telomeres have not been reported as having

any connection with SLE or other autoimmune diseases.

For better understanding of the present invention, together with other and further objects and the nature and advantages of the invention, reference is made to the following detailed description of specific embodiments.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the novel finding that autoantibodies (anti-DNA-antibodies) are recognized and bound by telomeres. In accordance with the present invention, a method is disclosed for the detection of autoantibodies or anti-DNA-antibodies in samples of eukaryotic origin, especially vertebrate body fluids, especially serum.

Body fluids which may contain anti-DNA-antibodies in addition to serum are, for instance, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, saliva, tears, urine, intestinal secretions, lymph fluid, etc. The IgG, IgM and IgA class antibodies are known to be secreted into the bloodstream, while mainly IgA antibodies are found in saliva, tears, etc. The preferred body fluid for quantitative detection according to the invention is mammalian serum.

Telomeres are evolutionally highly conserved in nature and all eukaryotic beings have telomeres of a very closely related structure. The vertebrate telomere is composed of repeated sequences of G- and C-rich complementary strands with a G-rich strand that extends past the rich strand as 3' overhang. Other eukaryotic telomeres have very similar sequences, usually only one nucleotide in the hexapeptide is different. Thus, the present invention is applicable on humans and animals alike.

It is also possible to use parts of the telomere that recognize the anti-DNA-antibodies as binding partners. Thus, a part of the hexanucleotide repeat, such as the TTAG, may be used for performing the recognition.

It has been found that the anti-DNA-antibodies in some samples may bind more specifically to one or the other of the complementary single-stranded sequences, while some bind more readily to a double-stranded telomeric DNA (dsDNA).

It is also possible to use specific antibodies to said telomeric sequence for competing with the binding reaction between telomere and sample anti-DNA-antibody. Specifically light chain fragments, Fabs of said specific antibodies may be used for competing with said anti-DNA-antibodies.

The telomeric sequence used in the present invention is either the single-stranded telomere sequence, which for vertebrates is 5'-TTAGGG-3', its complementary sequence (for vertebrates 5'-CCCTAA-3'), a functional part of any of the above, an oligonucleotide having one or more repeats of the telomeric or complementary sequence, a double-stranded DNA formed of the above single-stranded sequences or an oligonucleotide part thereof or repeats of the telomeric doublet.

In the method for detecting anti-DNA-antibodies according to the present invention, the selected functional telomeric sequence is synthesized in a way known to those skilled in the art, e.g. in commercial nucleotide synthesizers. The obtained sequence is then preferably immobilized on a carrier, which may comprise the walls of microtiter wells, glass or plastic beads, porous sheets, Sepharose etc. which carriers are well known to those skilled in the art.

Immobilization (solid-phase) techniques are also well known to those skilled in the art. The telomeric sequence may, for instance, be coated onto the walls of microtiter wells, glass or plastic beads, impregnated into porous sheets, filter papers or the like. The immobilization may be performed directly on the material in question or, preferably, by the use of a streptavidin-biotin system, which provides a stronger and more accurately oriented immobilization of the telomeric sequence. The telomeric sequences may be biotinylated either at the 3' or at the 5' end. However, the actual technique used for the immobilization is not critical to the performance of the invention. The amount of telomeric sequence used is between 7-2 $\mu\text{g/ml}$, or even considerably more. The amount of telomeric sequence is not critical to the performance of the assay. A preferred range is believed to be 100-500 ng/ml.

The specific telomeric antibody and the fragments thereof may be provided from the serum of patients suffering from autoimmune diseases with anti-DNA-antibodies in ways known to those skilled in the art, or they may be provided by recombinant DNA techniques. Thus, the telomeric sequences in

accordance with the present invention can be used for the production of anti-DNA antibodies and fragments thereof for therapeutic use.

When an immobilized telomeric sequence is contacted with a sample to be tested, any anti-DNA-antibodies present in the sample will attach to the immobilized sequence. The complex formed in the binding reaction may be detected by means well known in immunochemistry. Correspondingly, specific telomere antibodies or Fab fragments thereof may be used in a competitive immunochemical assay to indicate the presence of anti-DNA-antibodies in a sample.

The autoantibodies recognized by the telomeric sequences may be of different immunoglobulin classes, which may indicate so far unknown differences in the progression of the disease or disease pattern. Thus, the autoantibodies may be either IgG, IgM, IgA, IgD, or IgE class, since the anti-DNA-antibodies can belong to any of the known immunoglobulin classes.

Different anti-Ig antibodies will detect the presence of the different Ig class autoantibodies. Thus, for example, labeled anti-IgG will be used to determine the presence of the IgG class autoantibodies, anti-IgM antibody will be used for detecting the IgM class autoantibodies, and so on.

It is known in the art that SLE patients have predominantly IgG class autoantibodies, while for instance rheuma patients seem to have a predominance of IgM autoantibodies in their serum. However, the tests performed in connection with the present invention show that SLE patients often have either IgG, IgM or IgA class autoantibodies, the level of IgG class autoantibodies being elevated in 50 of 52 tested patients known to have SLE, compared to SLE negative patients. The two patients having rather low levels of IgG class antibodies were over 70 years of age and when IgG, IgM and IgA class antibodies were tested, all 52 SLE patients gave positive test results with the diagnostic test of the invention.

The bound anti-DNA-antibody can be detected with the aid of a label on an anti-Ig antibody used in a way known in the art, the sensitivity of the test depending on the label, the immunoassay, etc. The label may be a radioactive label, an enzyme label, a fluorochrome or fluorescence label, a dye, a sol, biotin, a luminescent label, a colored latex particle and/or a labeled polyclonal

or monoclonal antibody or the like. The telomeric sequence or a Fab or F(ab')₂ fragment may also be labeled and used in known types of competitive immunoassays. The assay may be an EIA, radioimmunoassay, immunofluorescent assay, immunoprecipitation, complement fixation, immunochromatographic assay or any other that is designed to detect autoantibody binding of a telomeric DNA.

The test may be formulated as a quantitative test such as an EIA assay or into a more or less qualitative rapid test, as desired, using procedures which are known *per se* in the art and which are exemplified in the Examples hereinafter.

The tests performed in connection with the present invention very conclusively show that a diagnosis of systemic lupus erythematosus patients can be performed using a telomeric sequence for the anti-DNA-antibody recognition. Out of 52 SLE patients all were diagnosed positive in at least one Ig class autoantibody binding when tested with the assays of the present invention. The tests of the present invention are easy to perform, they are quick and reliable. The need for this kind of test is enormous in the art, since previously the testing for SLE has been uncertain and tedious. In actual fact no certain diagnosis has been possible until clear signs of serious disorders have appeared.

The tests performed also show that patients with rheumatoid arthritis may be diagnosed by detecting the presence of anti-DNA-antibodies, especially in the IgM class.

Further, several psoriasis patients showed elevated levels of anti-DNA-antibodies compared to healthy controls.

In systemic lupus erythematosus (SLE) the serum of the patient almost invariably contains antibodies directed against the cell nuclei (anti-DNA-antibodies). Some of these are directly responsible for the disorders caused by the disease. According to the invention it has been found that telomeric sequences bind these autoantibodies. One aspect of the present invention therefore comprises the use of this binding affinity for removing anti-DNA-antibodies from body fluids, especially from the serum of humans.

The removal of anti-DNA-antibodies may be performed in an affinity column

containing immobilized telomeric sequences capable of retaining anti-DNA-antibodies from a fluid sample. The fluid may at need be recirculated through the column. After use the bound anti-DNA-antibodies may be removed from the column by means known *per se* and the column may be reused. The treated fluid, such as human serum may be returned in a continuous fashion to the patient or it may be used for other purposes.

Removal of anti-DNA-antibodies from a fluid may also be achieved by using various batch processes with the telomeric sequences immobilized on a matrix of a suitable kind, such as plastic beads, filters, etc.

The frequency and the length of time needed for treatment of, for instance, the serum of a human being suffering from SLE by an affinity column or other anti-DNA-antibody removal means, may be monitored with a test designed according to the present invention.

It has been found that the telomeric sequences bind to the anti-DNA-antibodies and neutralize their binding capacity and that, correspondingly, antibody fragments specific to said telomeric sequences compete with the binding of anti-DNA-antibodies to said telomeric sequences and hence prevent the anti-DNA-antibodies from being bound to telomeres.

The telomeric sequences, parts or repeats thereof, single or double-stranded, and antibody fragments used in the above described assays may thus also be used as active agents in drugs against the destructive activity of the anti-DNA-antibodies in patients suffering from diseases and/or disorders caused by or involving the presence of anti-DNA-antibodies.

More specifically, the active part of the drug is a single or double-stranded telomeric sequence, a repeat or a part thereof, a complementary sequence or a combination of any of the foregoing, (referred to generally as a telomeric sequence) which is recognized by a patient anti-DNA-antibody, or a fragment (Fab or F(ab')₂) of an antibody specific to any of said telomeric sequences. A drug according to the present invention thus comprises an amount of a telomeric sequence or antibody fragment, as defined above, which is effective to inhibit or reduce the destructive activity of the patient's anti-DNA-antibodies.

Special benefits are provided by using the above antibody fragments in drugs since they will compete with autologous anti-DNA-antibodies for binding to the patient's telomeric sequences. The Fabs and F(ab')₂ fragments lack effector functions and will not activate the complement cascade leading to cell destruction when bound to an antigen, as the whole anti-DNA-antibodies do.

The present invention comprises drugs for humans as well as veterinary preparations containing the vertebrate telomeric sequence.

Moreover, the present invention makes it possible to give the patient in question precisely the telomeric sequence or specific antibody fragment that will be most effective to prevent the binding of the patient's own anti-DNA-antibodies to telomeric sequences. The patient can first be tested with an assay according to the present invention so as to determine which telomeric sequence will best bind to the anti-DNA-antibodies. Thus, assays will determine whether single or double-stranded telomeric sequences should be used, and which Ig class the patient's anti-DNA-antibodies are.

In this therapeutic embodiment of the present invention the regimen for therapy is preferably designed individually to each patient based on their anti-DNA-antibody binding, i.e. which immunoglobulin class, IgG, IgM, IgA etc., and which telomeric form they prefer.

The amount of active telomeric agent that should be given to a patient will vary with the patient's race, age, illness, etc. and the most effective dose will have to be determined on a case by case basis. However, the telomeres are DNA sequences existing as such in the chromosomes and thus they are part of the mammalian body. Thus, it is not believed that the maximum amount of telomere given to a patient will be critical. In toxicity tests on rabbits no effect was observed after a telomere administration. The Fabs used according to the present invention will be of mammalian origin or they will be engineered by recombinant techniques to resemble the same and will therefore be acceptable to the mammalian body.

The active telomeric agent of the present invention may be formulated into pharmaceutical preparations such as injectable solutions in a way known in the pharmaceutical art.

The following Examples illustrate the invention without, however, limiting it in any way.

Example 1 Synthesis of telomeric sequences

Synthesis of oligonucleotides was carried out by a commercial apparatus, Oligonucleotide Synthesizer (Applied Biosystems). The following oligonucleotides were synthesized:

Order number 2667: 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3'

Order number 2668: 5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3'

A duplex DNA (2667 + 2668) was formed in the laboratory as follows:

A volume of 50 μ l (50 μ g) of each oligo (2667 and 2668) in 10 mM Tris- 1 mM EDTA containing 50 mM NaCl, pH 7.4 buffer were combined and heated for 5 min at 75 °C. The mixture was left to cool at room temperature for an hour. The cooled mixture was immediately coated on polystyrene microtiter wells (Nunc) in a concentration of 1 or 2 μ g per ml phosphate-buffered saline (PBS, pH 7.4). Each well received 100 μ l of coating solution.

Example 2 Assays for anti-DNA-antibody detection

1. Polystyrene microtiter wells were coated with the single-stranded oligonucleotides 2667 or 2668 or with the duplex DNA (2667 + 2668) obtained in Example 1. The coating concentration was 1 or 2 μ g per ml PBS, pH 7.4. The coating volume was 100 μ l. The plates were incubated at room temperature without cover until dry.

2. The plates were washed three times with 10 mM PBS, pH 7.4, containing 0.05% Tween 20 (Fluka AG).

3. Postcoating was carried out by applying a volume of 100 μ l of Dulbecco buffer containing 0.5% bovine serum albumin (BSA) per well to block the nonspecific binding. The incubation took one hour at 37°C under plastic

adhesive cover to prevent the differential evaporation.

4. The plates were washed as in 2, and airdried, and stored at +4 °C for later use.

Example 3

The detection of autoantibodies against telomeric sequences

1. Serum from diagnosed SLE patients and healthy controls, respectively, were diluted 1:10 in Dulbecco's buffer containing 0.5% BSA and 0.05% Tween 20 and a volume of 100 μ l of the serum dilution was incubated for two hours at 37°C in the coated wells under plastic adhesive cover.

2. The plates were washed three times with 10 mM PBS, pH 7.4, containing 0.05% Tween 20 (Fluka AG).

3. A volume of 100 μ l of alkaline phosphatase-labeled swine anti-human IgG (for IgG class autoantibody determination, Orion Diagnostica, Espoo, Finland) or alkaline phosphatase-labeled swine anti-human IgM (for IgM class autoantibody determination) diluted 1:200 in Dulbecco's buffer containing 0.5% BSA and 0.05% Tween 20.

The incubation took place for one hour at 37°C.

4. The plates were washed as in 2.

5. A volume of 100 μ l of substrate, 0.2% para-nitrophenyl phosphate disodium salt in diethanolamine buffer, pH 10.0, was added per well, and the plates were incubated for 30 min at room temperature.

6. A volume of 100 μ l of 1 M sodium hydroxide (NaOH) was added per well to stop the reaction.

7. The absorbances of the wells were recorded using a Titertek Multiscan spectrophotometer using the wavelength 405 nm.

The results of the test are shown in Tables 1 and 2.

TABLE 1: SLE-patient assays

Patient	IgG-class autoantibody			IgM-class autoantibody		
	2667	2668	2667 + 2668	2667	2668	2667 + 2668
1.	0.158 0.152	0.524 0.449	0.166 0.093	1.134 1.167	1.132 1.132	1.118 1.119
2.	0.095 0.095	0.268 0.237	0.132 0.119	0.017 0.012	0.782 0.700	0.119 0.110
3.	0.278 0.308	1.456 1.299	0.661 0.584	0.055 0.051	0.225 0.228	0.090 0.092
4.	0.434 0.419	0.699 0.700	0.185 0.165	0.058 0.057	0.200 0.190	0.113 0.106
5.	0.173 0.201	0.442 0.393	0.223 0.217	0.215 0.201	1.033 1.092	0.351 0.352
6.	0.077 0.088	0.833 0.615	0.276 0.248	0.045 0.041	0.330 0.299	0.161 0.160
7.	0.257 0.264	0.201 0.208	0.186 0.156	0.034 0.035	0.189 0.189	0.048 0.048
8.	0.090 0.106	0.058 0.083	0.042 0.030	0.039 0.040	0.116 0.093	0.037 0.033
9.	0.559 0.639	1.082 0.991	0.708 0.632	0.030 0.026	0.244 0.209	0.064 0.061
10.	0.093 0.104	0.069 0.065	0.035 0.029	0.038 0.029	0.253 0.225	0.079 0.074
11.	0.033 0.022	0.120 0.090	0.034 0.036	0.211 0.233	0.731 0.802	0.376 0.383
12.	0.359 0.394	0.908 0.852	0.284 0.262	0.020 0.038	0.219 0.251	0.083 0.079
13.	0.047 0.031	0.136 0.076	0.038 0.032	0.038 0.123	0.507 0.536	0.122 0.129
14.	0.061 0.046	0.184 0.137	0.059 0.054	0.163 0.168	1.317 1.573	0.396 0.416
15.	0.822	1.918	1.154	0.039 0.040	0.116 0.093	0.037 0.033
16.	0.086	0.628	0.073			
17.	1.794	1.832	1.597			

TABLE 2: Control assays (no SLE-diagnosis)

Patient	2667	IgG-class autoantibody		IgM-class autoantibody		2667 + 2668
		2668	2667 + 2668	2667	2668	
A.	0	0.001	0.004	0.014	0.146	0.058*
B.	0.024	0.005	0.002	0.006	0.056	0.023*
	0.001	0.018	0.004	0.016	0.074	0.039*
C.	0	0	0	0.005	0.121	0.058**
	0	0	0	0.021	0.165	0.078**
D.	0.002	0.002	0.005	0	0.042	0.025*
	0	0	0.002	0.006	0.060	0.047*
E.	0.002	0	0	0	0	0.008*
	0	0	0.002	0.009	0.025	0.018*
F.	0.001	0.003	0	0	0.050	0.029*
	0.002	0	0	0.003	0.082	0.033*
G.	0.005	0.003	0.002	0	0	0.004
	0.005	0	0.001	0.004	0.005	0.010

Note:

2667 and 2668 comprise single-stranded telomeric DNA

2667 + 2668 comprises double-stranded DNA

* Patients with suspected viral infection from the
diagnostic routine

** Patients C was born in 1992

The above results clearly show that the serum of SLE patients demonstrate a high level of anti-DNA-antibodies binding to the telomeric sequences while normal control individuals have a significantly lower level, almost negligible in the IgG class. The present assay can thus be used for screening SLE.

Example 4
Inhibition of anti-DNA-antibody binding to
immobilized telomeric sequences

To provide proof that the binding is specific and that the antibodies can be neutralized with the specific telomeric sequence, the assay was performed as described above in Example 3 with the following modification. An amount of 5 $\mu\text{g/ml}$ of oligonucleotide 2667, 2668 or the duplex DNA (2667+2668), respectively, was added to a 1:25 dilution of patient serum and incubated for one hour at room temperature. After this incubation a volume of 100 μl of each incubation was added per well coated with the identical telomeric sequence (as in Example 2). Incubation lasted for 2 hours at 37 °C, and the IgG or IgM class autoantibody binding was assayed using the corresponding labeled swine anti-IgG or anti-IgM antibodies, respectively, as in Example 3. The same patient serum diluted 1:25 without an addition of telomeric sequence served as a control.

The results are shown in Table 3.

TABLE 3

Inhibition of IgG class autoantibody binding to immobilized telomeric sequences by the telomeric sequence indicated in the Table.

SLE No.	Immobilized sequence 2667			
	without inhibitor	inh. 2667	inh. 2668	inh. 2667 + 2668
1.	1.840	0.037	0.151	0.035
	1.736	0.036	0.201	0.0221
2.	0.320	0.042	0.030	0.009
	0.300	0.045	0.048	0.029
	Immobilized sequence 2668			
	without inhibitor	inh. 2667	inh. 2668	inh. 2667 + 2668
1.	1.131	0.186	0.036	0.077
	0.963	0.101	0.022	0.075
2.	0.829	0.027	0.031	0.064
	1.035	0.034	0	0.039

Immobilized sequence 2667 + 2668				
	without inhibitor	inh. 2667	inh. 2668	inh. 2667 + 2668
1.	1.047 1.134	0.179 0.163	0.133 0.142	0.098 0.070
2.	0.223 0.270	0.042 0.045	0.027 0.040	0.031 0.031

Inhibition of IgM class autoantibody binding to immobilized telomeric sequences by the telomeric sequences indicated in the Table.

Immobilized sequence 2667				
SLE No.	without inhibitor	inh. 2667	inh. 2668	inh. 2667 + 2668
1.	0.244 0.214	0.078 0.061	0.046 0.029	0.029 0.051
2.	0.119 0.153	0.029 0.039	0.018 0.100	0.032 0.053

Immobilized sequence 2668				
	without inhibitor	inh. 2667	inh. 2668	inh. 2667 + 2668
3.	0.487 0.489	0.119 0.139	0.071 0.011	0.170 0.125
4.	0.289 0.278	0.053 0.147	0 0	0.073 0.073

Immobilized sequence 2667 + 2668				
	without inhibitor	inh. 2667	inh. 2668	inh. 2667 + 2668
1.	0.355 0.292	0.101 0.106	0.059 0.049	0.078 0.097
2.	0.074 0.075	0.121 0.068	0.020 0.064	0.012 0.060

The above test results show that the telomeric sequences are capable of binding to anti-DNA-antibodies present in serum in vitro. This clearly implies that said telomeric sequences are capable of inhibiting the telomere-specific anti-DNA-antibody binding site also in vivo. The telomeric sequences are therefore proposed for being used as specific therapeutic agents in patients suffering from autoimmune diseases which include the presence of anti-DNA-antibodies in the body fluid of said patient.

Example 5

Inhibition of IgG class autoantibody binding to parts of the telomeric sequence

The test of Example 4 was repeated for the partial sequences GGG and CCC, respectively. The results are shown in the Table below.

Immobilized sequence 2667			
Patient no.	without inhibitor	inh. GGG	inh. CCC
3.	0.600	0.528	0.245
4.	0.327	0.281	0.198

Immobilized sequence 2668			
Patient no.	without inhibitor	inh. GGG	inh. CCC
3.	0.765	0.734	0.528
4.	0.943	0.875	0.807

Patient no.	Immobilized sequence 2667 + 2668		
	without inhibitor	inh. GGG	inh. CCC
3.	0.372	0.323	0.323
4.	0.147	0.121	0.143

The results indicate that some binding also takes place to parts of the telomeric sequence.

Example 6

Immobilization of streptavidin-biotinylated telomeric sequences

1. Preparation of streptavidin-coated plates, strips, Latex particles and filter paper

A volume of 100 μ l of purified streptavidin (1.25-10 μ g/ml), purchased from Algal Oy, Finland, was immobilized in 0.01 M PBS (phosphate-buffered saline), pH 7.4, at room temperature. The unbound streptavidin was removed by three washes with the washing buffer 10 mM PBS containing 0.15 M NaCl and 0.05% Tween 20 (Fluka AG, Germany), air-dried and stored at +4 °C, if not immediately used. A coating concentration of 2 μ g/ml was later used for the biotinylated oligonucleotide binding.

2. Synthetic telomeric sequences

Biotinylated synthetic oligonucleotides were purchased from Pharmacia, Sweden. The following oligonucleotides were synthesized:

EMS 1: 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3'-biotin

EMS 2: 5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3'-biotin

SME 1: biotin-5'-TTAGGG-TTAGGG-TTAGGG-TTAGGG-TTAGGG-3'

SME 2: biotin-5'-CCCTAA-CCCTAA-CCCTAA-CCCTAA-CCCTAA-3'

EMS 1+2 and SME 1+2: Duplex DNAs (EMS 1+2 and SME 1+2) were prepared in the laboratory as follows:

A volume of 50 μ l (50 μ g) of each oligo (EMS 1 and EMS 2, and SME 1 and SME 2) in 10 mM Tris-1 mM EDTA containing 50 mM NaCl, pH 7.4, buffer were combined and heated for 5 min at 75 °C. The mixture (EMS 1+2, or SME 1+2) was left to cool at room temperature for an hour. The cooled mixture (100 μ l) was immediately diluted (200 ng/ml) in a binding buffer 0.01 M PBS containing 0.15 NaCl, 0.05% Tween 20 and 0.5% bovine serum albumin (BSA) and let to bind the immobilized streptavidin for 2 h at 37 °C. The same binding (or incubation) buffer was used in complex formation of streptavidin-EMS 1 (or -SME 1) and streptavidin-EMS 2 (or -SME 2) complexes.

The solid-phase bound streptavidin-oligonucleotide complexes, after three washes with the above washing buffer, were used for anti-DNA-antibody detection. The solid-phase complexes were stored for two months at room temperature, +4 °C and -20 °C without statistically significant loss of binding activity.

The concentration of oligonucleotides worked well in the range of 7-500 ng/ml, but the concentration of 200 ng/ml was selected for anti-DNA-antibody detection. Maximum binding was obtained already in 30 min at +37 °C.

Example 7

Detection of autoantibodies against telomeric sequences

1. Serum (and cerebrospinal fluid) from diagnosed systemic lupus erythematosus (SLE), rheumatoid arthritis and psoriasis patients as well as healthy controls, respectively, were diluted 1:50 in the binding buffer of Example 6 containing 0.5% BSA and 0.05% Tween 20. A volume of 100 μ l of the serum dilution was applied per well containing streptavidin-telomere complexes, and incubated for one hour at +37°C (or at room temperature) under plastic adhesive cover.

2. The wells were washed three times with 10 mM PBS, pH 7.4, containing 0.05% Tween 20.

3. A volume of 100 μ l of alkaline phosphatase-labeled swine anti-human IgG (gamma-chain specific), IgM (μ -chain specific) or IgA (alfa-chain specific) conjugate (Orion Diagnostica, Espoo, Finland) diluted 1:200 in the 10 mM PBS, pH 7.4 containing 0.5% BSA and 0.05% Tween 20.

The incubation took place for one hour at 37 °C.

4. The wells were washed as in 2.

5. A volume of 100 μ l of substrate, 0.2% para-nitrophenyl phosphate disodium salt in diethanolamine buffer was added per well, and the plates were incubated for 30 min at room temperature.

6. A volume of 100 μ l of 1 M sodium hydroxide (NaOH) was added per well to stop the reaction.

7. The absorbances of the wells were recorded using a Titertek Multiskan spectrophotometer (Flow Laboratories, U.K.) using the wavelength 405 nm.

Some of the results of the test are indicated in Tables 4, 5, 6 and 7, which show mean values of representative ones of the performed duplicate tests. In the Tables W stands for woman and M stands for male, the figures in connection therewith indicating the patients age.

The Tables show that SLE and RA patients have an elevated level of anti-DNA-antibodies compared to the healthy controls. Some psoriasis patients also have elevated levels of anti-DNA-antibodies. The healthy controls had clearly lower absorbance levels. The cut-off value the absorbance at 405 nm for the assay of the present test is 0.150 ($A_{405} = 0.150$). Levels elevated above 0.150 absorbance were obtained primarily in the IgG class in SLE patient sera and in the IgM class in RA patient sera.

TABLE 4

SLE-patient assays

Patient	IgG-class autoantibody			IgM-class autoantibody			IgA-class autoantibody		
	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2
1. W 39	0.437	0.336	0.177	0.172	0.365	0.253	0.1121	0.144	0.140
2. W 32	1.012	1.306	0.645	0.194	0.383	0.277	0.357	0.429	0.361
3. W 42	0.295	0.202	0.198	0.579	0.731	0.738	0.165	0.175	0.147
4. W 46	0.954	0.673	0.867	0.253	0.276	0.274	0.377	0.286	0.322
5. M 28	1.317	1.408	1.071	0.157	0.219	0.167	0.335	0.301	0.244
6. W 31	0.767	0.801	0.733	0.102	0.123	0.091	0.224	0.176	0.168
7. W 18	1.666	1.030	1.592	0.336	0.312	0.381	0.387	0.279	0.366
8. M 28	0.823	0.922	0.700	0.118	0.148	0.096	0.330	0.281	0.260
9. W 35	1.070	1.085	0.712	0.246	0.247	0.265	0.400	0.373	0.330
10. W 36	0.563	0.462	0.367	0.114	0.141	0.090	0.296	0.235	0.206

TABLE 5

Rheumatoid arthritis (RA) patient assays

Patient	IgG-class autoantibody			IgM-class autoantibody			IgA-class autoantibody		
	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2
1. W 62	0.167	0.136	0.174	0.450	0.273	0.305	0.229	0.258	0.207
2. W 64	0.089	0.128	0.107	0.171	0.170	0.115	0.971	0.522	0.728
3. W 34	0.159	0.174	0.180	0.260	0.372	0.295	0.089	0.075	0.012
4. W 41	0.135	0.205	0.121	0.495	0.653	0.653	0.082	0.109	0.081
5. M 70	0.293	0.283	0.323	0.543	0.567	0.398	0.367	0.376	0.454
6. W 43	0.068	0.064	0.071	0.066	0.276	0.343	0.114	0.152	0.168
7. W 43	0.640	0.585	0.410	0.520	0.558	0.556	0.248	0.204	0.218
8. W 68	0.064	0.154	0.122	0.455	0.431	0.424	0.183	0.197	0.206
9. W 43	0.586	0.925	0.502	0.615	0.896	0.856	0.262	0.363	0.406
10. W 48	0.238	0.129	0.169	0.373	0.585	0.583	0.135	0.161	0.124

TABLE 6

Control assays (no SLE or RA diagnostic)

Healthy controls

P	IgG-class autoantibody			IgM-class autoantibody			IgA-class autoantibody		
	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2
1.	0.063	0.095	0.053	0.066	0.066	0.052	0.067	0.102	0.066
2.	0.039	0.042	0.019	0.110	0.157	0.146	0.098	0.099	0.094
3.	0.175	0.102	0.146	0.108	0.141	0.124	0.059	0.079	0.045
4.	0.090	0.078	0.050	0.137	0.155	0.213	0.099	0.065	0.068
5.	0.098	0.090	0.048	0.124	0.155	0.135	0.079	0.046	0.061
6.	0.067	0.092	0.015	0.074	0.114	0.116	0.104	0.115	0.107
7.	0.058	0.031	0.027	0.076	0.098	0.090	0.141	0.066	0.107
8.	0.079	0.040	0.050	0.120	0.168	0.219	0.074	0.119	0.156
9.	0.052	0.041	0.044	0.079	0.126	0.166	0.105	0.095	0.119
10.	0.073	0.045	0.031	0.137	0.155	0.165	0.086	0.123	0.139

TABLE 7

Assays on psoriasis patients

Patient	IgG-class autoantibody			IgM-class autoantibody			IgA-class autoantibody		
	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1+2
1. W 51	0.467	0.342	0.402	1.609	>2.000	1.854	0.671	0.700	0.617
2. M 40	0.136	0.020	0.110	0.279	0.288	0.340	0.097	0	0.123
3. M 52	0.173	0.109	0.134	0.322	0.164	0.227	0.242	0.111	0.129
4. W 51	0.361	0.294	0.358	1.168	1.430	1.596	0.200	0.185	0.196
5. M 47	0.171	0.090	0.142	0.238	0.122	0.141	0.220	0.116	0.145
6. M 54	0.129	0.127	0.120	0.154	0.240	0.160	0.156	0.177	0.120
7. M 49	0.148	0.061	0.096	0.115	0.146	0.146	0.119	0.068	0.096
8. M 38	0.238	0.093	0.198	0.208	0.131	0.208	0.008	0	0.006
9. W 48	0.226	0.125	0.137	0.253	0.213	0.143	0.125	0.032	0.058
10. W 31	0.094	0.044	0.073	0.163	0.143	0.148	0.218	0.106	0.103

Example 8
Latex agglutination test

1. A volume of 50 μ l of polystyrene Latex beads (Sigma, St. Louis, U.S.A) with a particle diameter 1.1 μ was incubated in 10 mM PBS, pH 7.4 at a concentration of 5 μ g streptavidin/ml, overnight at room temperature.
2. Any unbound streptavidin was removed from the bound by washing with 0.01 M PBS, pH 7.4, followed by centrifugation.
3. A volume of 50 μ l of biotinylated oligonucleotide EMS 1 of Example 6 (5 μ g/ml in mM PBS, pH 7.4, containing 0.05% Tween and 0.5% BSA) was incubated with streptavidin-coated Latex particles.
4. Any unbound oligonucleotides were removed from the bound by washing with 0.01 M PBS, pH 7.4, followed by centrifugation.
5. Sera from SLE patients were diluted 1:5 with incubation buffer (glycine 7.5 g/l supplemented with NaCl 10 g/l, pH 8.2).
6. A drop (50 μ l) of patient serum dilution was incubated with a drop of coated Latex particles.
7. With anti-DNA positive sera a visible agglutination of Latex particles occurred, whereas the negative sera gave no reaction.

The test provides a rapid qualitative test for the diagnosis of SLE.

Example 9
Complement fixation test

1. Anti-DNA-antibody positive SLE sera and control sera, respectively, were serially diluted in veronal buffer (also called barbital buffer), pH 7.6. A volume of 25 μ l of diluted specimens were transferred to the U-shaped microtiter wells.

2. The unlabeled telomeric sequence, 2667, 2668 or 2667+2668, respectively, as defined in Example 1 was added into the serum dilution at a concentration of 10 $\mu\text{g/ml}$, and a volume of 25 μl was added per sample well.

3. Guinea pig complement (25 μl) was added per well overnight.

4. In the morning a haemolytic system (25 μl of sheep red cell suspension and 25 μl of rabbit anti-sheep antibodies) was applied into the sample wells. The reaction mixtures were shaken and then incubated for 30 min at 37 °C, shaken again and reincubated for 30 min at 37 °C.

5. The results were visualized both objectively and by the inhibition of a light path through the reaction mixture, as measured with a spectrophotometer. A total haemolysis (the disruption of cells) was obtained with sera without antibodies to the antigen (in this case the telomeric sequences) tested. The presence of anti-DNA-antibodies in the serum specimens tested resulted in a visible red pellet in the corresponding reaction well (based on the consumption of the complement in the reaction system).

The complement fixation test provides a rapid qualitative test for the diagnosis of anti-DNA-antibodies in a sample.

Example 10

Detection of anti-telomere antibodies using immunofluorescence antibody technique

1. The metaphase chromosomes of three day-cultures of blood lymphocytes, fixed in glass slides, were obtained from the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital.

2. Serum samples with and without anti-DNA antibodies, respectively, were diluted 1:20 in 0.01 M PBS, pH 7.4, containing 0.5% BSA (bovine serum albumin) and incubated on the slides in a humid chamber for 30 min at +37 °C.

3. The slides were washed three times for 5 min at room temperature, and air-dried.

4. The slides were incubated with 1:10 dilution of FITC (fluorescein isothiocyanate)-conjugated goat anti-human IgG antibodies (Kallestad, Austin, Texas 78746, USA) for 30 min in a humid chamber at + 37 °C.

5. The slides were washed as in 3) and air-dried.

6. A mounting medium and cover slides were added and the slides were ready for microscopic examination.

Results:

The anti-DNA sera gave a positive speckled-like fluorescent staining (a typical telomere staining) with 1:50 magnification and oil, whereas with anti-DNA negative sera no staining was seen.

The procedure provides a rapid test for detecting anti-DNA-antibodies in a sample.

Example 11

Rapid detection of anti-DNA-antibodies by labeled anti-Ig

1. A few drops of a telomeric sequence according to Example 1 and Example 6, respectively, are added to pieces of filter paper.

2. A drop of patient serum or a drop of spit (saliva), respectively, is added onto the drops of telomere.

3. The filters are rinsed to remove any unbound fluid.

4. A drop of labeled anti-human IgG or labeled anti-human IgA, respectively, is added to the filters at the previous drop sites.

5. In case of the presence of anti-DNA-antibodies in the sample drops, the label gives a visible positive reading when using a color-forming or precipitating substrate.

The above test provides a crude but a very cheap, simple and quick detection of anti-DNA-antibodies in body fluids without the need for any special equipment.

Example 12

Use of differential antibody avidity (affinity) in single- or double-stranded DNA binding

Four patient sera of typical SLE cases were selected for the test. The patients were 16 (P16), 17 (P17), 38 (P38) and 63 (P63) years old. The test was performed as described in Example 7, in parallel with the otherwise similar experiment, with the exception that after the serum incubation the bound DNA antibodies were washed three times for 5 min at room temperature with freshly prepared 8 M urea diluted in 10 mM PBS, pH 7.4, containing 0.05% Tween 20. This treatment is known to release the low avidity antibodies from the antigen but has no or only a minor effect on the high avidity antibodies.

The results showed that the anti-DNA antibodies had a different and partly age-related affinity for telomeres. Three of the four patients showed a low avidity anti-DNA binding to the single-stranded DNA (EMS 1 or EMS 2), since the bound antibodies (P16, P38 and P63) could be removed with 8 M urea. The avidity of antibodies to the duplex DNA (EMS 1 + 2) was higher, since only in two cases the bound anti-DNA-antibodies (P38 and P63) were totally removed with 8 M urea.

The test can be used to indicate the length of time that the patient has been exposed to anti-DNA-antibodies. A prolonged disease and a consequent long exposure time leads to anti-DNA-antibodies having a higher affinity to the telomeric sequence. The age of the patient should generally also be taken into account, however, since the amount of telomeres is known to be related to the age of a person.

Example 13

Storage of coated streptavidin-telomere complexes at different temperatures

The capacity (or activity) of streptavidin-telomere complexes to bind anti-DNA-antibodies from the patient serum was tested after the plates coated with streptavidin-telomere (EMS 1, EMS 2 or EMS 1+2 of Example 6) were incubated at +37°C, at room temperature, at + 4°C or at -20°C for one day (the baseline), two weeks, one month or two months. In each case the test was performed using the same patient serum (1:50 dilution), the same conjugate (1:200 dilution of alkaline phosphatase labelled swine anti-human IgG) and the same buffers and reagents as described in Example 7 for the detection of autoantibodies against telomeric sequences. Each measurement was performed in triplicate wells.

The results show that no binding activity of the coated complexes was lost during the storage at +4°C or -20°C in two months. A 20% decrease in the absorbance value (directly proportional to the binding activity of coated complexes) was obtained after storage at room temperature, and a 30% decrease at +37°C, compared to the baseline value.

Example 14 a
Anti-DNA-antibody separation column
using telomere affinity column

1. Plastic disposable columns with a 4 μ filter plug were packed with suspension of polystyrene Latex particles (Serva, Heidelberg, Germany) with a particle size 1.1 μ .
2. Purified streptavidin at a concentration of 2 μ g/ml PBS, pH 7.4, was added into the column, and incubated with the suspension in a roller overnight at room temperature.
3. Any unbound streptavidin was removed by centrifugation, and the streptavidin-coated particles were washed ten times with PBS, pH 7.4.
4. The biotinylated telomeric sequences EMS 1, EMS 2 or EMS 1+2 of Example 6 at a concentration of 2 μ g/ml were incubated with the streptavidin-coated Latex particles for 2 hour in a roller at room temperature.

5. Any unbound telomeric sequences were removed by washing the column ten times with PBS.
6. The anti-DNA sera of patients with SLE were applied on the column, incubated for 5 min in the column at room temperature, and then passed through the column.
7. The column was washed ten times with PBS, pH 7.4, and the telomeric sequence-bound anti-DNA-antibodies were eluted using a 5 min repetitive incubation of 0.1 M glycine, pH 3, in a roller, followed by centrifugation. A volume of 200 μ l per fraction after each incubation was collected, followed by adjustment of the pH to 7.4.
8. The results clearly show that anti-DNA-antibodies bind to the telomere affinity column. With use of small columns (0.4 ml of affinity matrix) and undiluted anti-DNA-antibody specimens, about a half of the antibodies originally present in the patient serum (A405 0.963-1.420) were bound to the column (A405 0.444-0.670). With an increase of the column size or by recycling the column, all anti-DNA-antibodies that are specific to telomeric sequences are bound, and will be removed from the sera.

Example 14 b

Anti-DNA-antibody removal in batch separation

The removal of anti-DNA-antibodies from the sera of Example 13a was carried out also by the use of a batch separation system, where the affinity column was omitted. In this case the affinity matrix was incubated with the anti-DNA sera, followed by centrifugation and/or direct filtration of the serum through the 0.22 μ m filter column.

Removal of anti-DNA-antibodies was successfully carried out also in this way.

Example 15

Toxicity test of telomeric sequences *in vivo*

Each rabbit (8 weeks old) received intravenously 100 μ g of the sequences EMS 1, EMS 2 or EMS 1 + 2 of Example 6 in complete Freund's adjuvant three

times at ten days' intervals. The rabbits were examined daily for two months for their physical status, which was found to be normal.

The detection of antibodies against telomeric sequences was performed similarly as in humans, except for the conjugate which was alkaline phosphatase-labeled swine anti rabbit IgG. No antibodies against telomeric sequences were detected in the sera of the rabbits.

Example 16

Purified human F(ab')₂ or Fab antibodies
to telomeric sequences as therapeutic agents

Purification of human IgG from a patient serum containing anti-DNA-antibodies

1. Human IgG from a serum of a patient with SLE was purified using a ProSep^{RA} high capacity matrix (Bioprocessing, Co Durham, England), which consists of protein A-coated glass beads with a binding capacity of 40 mg IgG/ml beads.

This method can also be used to absorb the IgG-class anti-DNA-antibodies from patient serum.

2. The serum was passed through the column, and the column washed until A₂₈₀=0 in the flow-through.

3. The bound IgG was eluted from the column with 0.1 M glycine, pH 3.0. The fractions containing IgG were pooled, neutralized with 1 M Tris buffer, dialyzed against PBS, pH 7.4, overnight, and measured for the protein content.

Purification of F(ab')₂ antibodies from purified IgG

1. In the purification of F(ab')₂ fragments from the purified IgG, the Immuno PureR F(ab')₂ Preparation kit (Pierce, no. 44888, Rockford, Illinois 61105, USA) was used according to manufacturer's instructions. In principle, the crude pepsin-IgG digest is passed through the protein A-column, where the whole IgG and the Fc part of IgG are bound, but not the F(ab')₂ fragments, which elute from the column, are pooled and dialyzed against PBS, pH 7.4.

Result:

The purified F(ab')₂ antibodies bound the immobilized telomeric sequences in a dose-dependent fashion, as detected in EIA using alkaline phosphatase-conjugated goat anti-human IgG, F(ab')₂, (Pierce, Rockford, Illinois, 61105, USA) for the telomere-bound F(ab')₂ antibody detection. The result shows that F(ab')₂ antibodies bind telomeric sequences. They can be purified to the monospecificity using a telomere affinity chromatography. Being of human origin they can be safely used intravenously as competitors for autologous IgG-class anti-DNA antibodies for binding telomeric sequences. The F(ab')₂ antibodies lack effector functions and do not activate the complement cascade when bound to an antigen.

The treatment of F(ab')₂ antibodies with reducing agents results in the formation of monomeric Fab antibodies, which are the biological form of the recombinant Fabs prepared using phage display systems. The monomeric Fabs, whether of biological origin or preferably produced by recombinant DNA technology, may be used intravenously as therapeutic agents the same way as the F(ab')₂ for competing with and thus preventing the destructive action of whole anti-DNA-antibodies in patient body fluids.

Example 17**The subclasses of the IgG-class antibodies
recognized by telomeric sequences**

The test was performed as described in Example 7, with the exception that alkaline-phosphatase-labeled mouse anti-IgG1, IgG2, IgG3 or IgG4 antibodies (Zymed Laboratories, Inc, Calif. USA) were used to detect the subclass-specificity of IgG anti-DNA-antibody.

The results of 20 tested sera from patients with SLE show that the anti-DNA autoantibodies recognized by telomeric sequences EMS1, EMS2 or EMS1 + 2 are primarily IgG1-class and to some extent IgG2-class antibodies.

TABLE 8

Patient	IgG1-class autoantibody		IgG2-class autoantibody		IgG3-class autoantibody		IgG4-class autoantibody	
	EMS1	EMS2	EMS1+2	EMS1	EMS2	EMS1	EMS2	EMS1+2
1.	0.604	0.855	0.540	0.128	0.118	0		0
2.	0.537	0.964	0.6021	0.315	0.262	0		0
3.	1.847	>2.000	>2.000	0.373	0.435	0		0
4.	0.225	0.332	0.285	0.189	0.131	0		0
5.	0.374	0.342	0.303	0.035	0.044	0		0
6.	0.336	0.364	0.405	0.037	0.042	0		0
7.	0.160	0.902	0.420	0.166	0.181	0		0
8.	0.128	0.200	0.226	0.031	0.054	0		0
9.	0.174	0.291	0.271	0.043	0.053	0		0
10.	0.058	0.110	0.091	0.036	0.065	0		0
11.	0.219	0.295	0.320	0.070	0.059	0		0
12.	0.121	0.106	0.141	0.078	0.095	0		0
13.	0.149	0.282	0.201	0.077	0.078	0		0
14.	0.228	0.591	0.340	0.117	0.046	0		0
15.	0.164	0.229	0.238	0.060	0.052	0		0
16.	>2.000	1.418	>2.000	0.379	0.222	0		0
17.	0.629	0.374	0.513	0.042	0.051	0		0
18.	0.195	0.469	0.308	0.044	0.140	0		0
19.	0.339	0.352	0.566	0.067	0.063	0		0
20.	>2.000	1.850	1.881	0.195	0.458	0		0

Example 18

Pharmaceutical preparation

A pharmaceutical preparation is prepared from the telomeric sequence 2667: 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3' by dissolving 1 μ g/ml of said sequence in physiological buffer. The solution is stored at 4 °C under sterile conditions.

A second pharmaceutical preparation is prepared in the corresponding way from the IgG class Fab antibodies of Example 16.

Example 19

Administering a telomeric sequence or Fab to SLE patients

The serum from SLE patients is diagnosed with an assay according to the invention. Said serum is found to contain an elevated amount of anti-DNA-antibodies of the IgG class binding predominantly to the telomeric sequence 2667 of Example 1.

The pharmaceutical preparations of Example 18 are administered to said patients intravenously at a dose of 100 ml three times in three weeks. After the administration period the serum of said patients is again tested in an assay according to the invention and the absorbance levels are found to be reduced from the original ones due to a binding of the telomeres or Fabs, respectively, to anti-DNA-antibodies circulating in the blood-stream.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Salonen, Eeva-Marjatta
(B) STREET: Tunturikatu 15 B
(C) CITY: Helsinki
(E) COUNTRY: Finland
(F) POST CODE (ZIP): 00100

(ii) TITLE OF INVENTION: Diagnostic Method, Test Kit, Drug and
Therapeutic Treatment for Autoimmune Diseases

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Borenius & Co Oy Ab
(B) STREET: Arkadiankatu 21 A
(C) CITY: Helsinki
(E) COUNTRY: Finland
(F) POST CODE (ZIP): 00100

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +358-0-492593
(B) TELEFAX: +358-0-493542
(C) TELEX: 124260 boco fi

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTAGGGTTAG GGTAGGGTT AGGGTTAGGG

30

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCCTAACCCT AACCTAACC CTAACCCTAA

30

CLAIMS

1. A method for detecting anti-DNA-antibodies in a sample characterized in contacting said sample with a telomeric sequence of DNA selected from the group consisting of a single-stranded telomeric sequence, a complementary strand thereto, a double-stranded telomere, and a part or a repeat or a combination of any of the foregoing, to provide binding of said antibodies to said telomeric sequence of DNA.
2. The method according to claim 1, wherein the telomeric sequence is selected from the group consisting of the vertebrate telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double-stranded vertebrate telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.
3. The method according to claim 2, wherein said repeat of said telomere strand is selected from the group consisting of
5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3'
5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3', and
a duplex thereof.
4. The method of claim 1 wherein an antibody to said telomeric sequence or a fragment of said antibody, especially a F(ab')₂ or a Fab fragment, is used in said detection.
5. The method of claim 4, wherein the immunoglobulin (Ig) class of said anti-DNA-antibodies is determined with the aid of anti-Ig-antibodies, especially anti-IgG, anti-IgM or anti-IgA antibodies.
6. The method of claim 5, wherein the immunoglobulin subclass of said anti-DNA-antibodies is determined with the aid of specific subclass anti-Ig-antibodies, especially anti-IgG1 or anti-IgG2 antibodies.
7. The method according to claim 1 wherein said sample is vertebrate body fluid.
8. The method according 7, wherein the body fluid is human serum or cerebrospinal fluid.

9. A diagnostic method for detecting autoimmune diseases in vertebrates characterized in contacting a body fluid sample from said vertebrate with a vertebrate telomeric sequence of DNA selected from the group consisting of a single-stranded vertebrate telomeric sequence, a complementary strand thereto, a double-stranded vertebrate telomere, a part or a repeat or a combination of any of the foregoing, to detect an elevated level of autoantibodies present in said body fluid.
10. The diagnostic method of claim 9 wherein said autoimmune disease is selected from the group consisting of Lupus Erythematosus, Rheumatoid Arthritis and Scleroderma.
11. The diagnostic method of claim 10 wherein said autoimmune disease is Systemic Lupus Erythematosus (SLE).
12. The diagnostic method according to claim 9, wherein said telomeric sequence is selected from the group consisting of the vertebrate telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double-stranded vertebrate telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.
13. The diagnostic method according to claim 12, wherein said repeat of said telomere strand is selected from the group consisting of
5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3'
5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3', and
a duplex thereof.
14. The diagnostic method of claim 9, wherein an antibody or a fragment of an antibody to said telomeric sequence, especially a F(ab')₂ or a Fab fragment, is used in said detection.
15. The diagnostic method of claim 14, wherein the immunoglobulin (Ig) class of said anti-DNA-antibodies is determined with the aid of anti-Ig-antibodies, especially anti-IgG, anti-IgM or anti-IgA antibodies.
16. The diagnostic method according to claim 15, wherein SLE in a human patient is detected by contacting a sample of said patient's body fluid with an

immobilized vertebrate telomeric sequence to bind anti-DNA-antibodies present in said fluid to said immobilized telomeric sequence and detecting an elevated level of bound IgG class autoantibodies among said bound anti-DNA-antibodies.

17. The diagnostic method according to claim 15, wherein Rheumatoid Arthritis in a human patient is detected by contacting a sample of said patient's body fluid with an immobilized vertebrate telomeric sequence to bind anti-DNA-antibodies present in said serum to said immobilized telomeric sequence and detecting an elevated level of bound IgM class autoantibodies among said bound anti-DNA-antibodies.

18. A test kit for the detection of anti-DNA-antibodies, especially in the diagnosis of autoimmune diseases in vertebrates, characterized in that said test kit includes a vertebrate telomeric sequence of DNA selected from the group consisting of a single-stranded vertebrate telomeric sequence, a complementary strand thereto, a double-stranded vertebrate telomere, a part or a repeat or a combination of any of the foregoing, capable of binding anti-DNA-antibodies present in a body fluid of said vertebrate.

19. The test kit according to claim 18, which additionally includes a label capable of indicating the binding of or inhibition of binding of said anti-DNA-antibody to said sequence.

20. The test kit of claim 19, which includes an antibody or a fragment of an antibody to said telomeric sequence, especially a F(ab')₂ or a Fab fragment, for use in said detection.

21. The test kit of claim 20, which includes specific anti-Ig-antibodies, especially anti-IgG, anti-IgM or anti-IgA antibodies for use in determining the immunoglobulin (Ig) class of said anti-DNA-antibodies.

22. The test kit of claim 21, which includes specific subclass anti-Ig-antibodies, especially anti-IgG1 or anti-IgG2 antibodies for the detection of immunoglobulin subclasses of said anti-DNA-antibodies.

23. The test kit according to claim 19 wherein said autoimmune disease is

selected from the group consisting of Lupus Erythematosus, Rheumatoid Arthritis and Scleroderma.

24. The test kit according to claim 23 wherein said autoimmune disease is Systemic Lupus Erythematosus (SLE).

25. The test kit according to claim 19 wherein said telomeric sequence is selected from the group consisting of the vertebrate telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double-stranded vertebrate telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.

26. The test kit according to claim 25, wherein said repeat of said telomere strand is selected from the group consisting of
5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3'
5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3', and
a duplex thereof.

27. The test kit according to claim 19, wherein said telomeric sequence is immobilized on a solid support.

28. The test kit according to claim 27, wherein said telomeric sequence is biotinylated at the 3' or the 5' end and is immobilized on a streptavidin-coated support.

29. The test kit according to claim 20, wherein said label is provided on a telomere-specific anti-Ig-antibody, on a fragment thereof or on a second telomeric sequence.

30. The test kit according to claim 20 wherein said label is selected from the group consisting of a radioactive label, an enzyme label, a fluorochrome or fluorescence label, a dye, a sol, biotin, a luminescent label, and a colored particle such as a colored latex particle.

31. The test kit according to claim 20, which includes an amount of one or more labeled anti-Ig-antibodies selected from the group consisting of anti-IgG, anti-IgM, anti-IgA, anti-IgD, and anti-IgE antibodies or subclasses thereof.

32. The test kit according to claim 31, wherein said anti-Ig-antibody is selected from the group consisting of anti-IgG, anti-IgM, anti-IgA, anti-IgG1 and anti-IgG2 antibodies.
33. The text kit according to claim 27 or 28, wherein said telomeric sequence is immobilized on the walls of microtiter wells, on Sepharose, on glass or plastic beads or on a porous sheet.
34. A solid phase means for the removal of anti-DNA-antibodies from a fluid sample, characterized in that it includes an immobilized telomeric sequence of DNA selected from the group consisting of a single-stranded telomeric sequence, a complementary strand thereto, a double-stranded telomere, a part or a repeat or a combination of any of the foregoing, capable of binding anti-DNA-antibodies present in a fluid.
35. The solid phase means according to claim 34 wherein said telomeric sequence is selected from the group consisting of the vertebrate telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double-stranded vertebrate telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.
36. The solid phase means according to claim 35, wherein said repeat of said telomere strand is selected from the group consisting of
5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3'
5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3', and
a duplex thereof.
37. The solid phase means according to claim 34, wherein said means is in the form of a affinity column packed with solid material, such as glass or plastic beads or a porous material, including immobilized telomeric sequences.
38. The solid phase means according to claim 37, wherein said telomeric sequence is biotinylated at the 3' or the 5' end and is immobilized on a strept-avidin-coated support.
39. A therapeutic method for treating vertebrates suffering from an autoimmune disease, characterized in directing a body fluid of said vertebrate through a column or matrix containing immobilized telomeric sequences

selected from the group consisting of a single-stranded vertebrate telomeric sequence, a complementary strand thereto, a double-stranded vertebrate telomere, and a part or a repeat or a combination of any of the foregoing to remove anti-DNA-antibodies from said body fluid.

40. A drug for patients suffering from autoimmune disorders, characterized in that said drug contains an amount of telomeric sequences or fragments of antibodies thereto effective in inhibiting or competing with anti-DNA-antibodies present in the body fluid of said patient from binding to the DNA of said patient, said telomeric sequence being selected from the group consisting of a single-stranded vertebrate telomeric sequence, a complementary strand thereto, a double-stranded vertebrate telomere, and a part or a repeat or a combination of any of the foregoing, said telomeric sequence being included in a preparation with an acceptable pharmaceutical carrier.

41. The drug according to claim 40 wherein said autoimmune disease is selected from the group consisting of Lupus Erythematosus, Rheumatoid Arthritis and Scleroderma.

42. A therapeutic method for treating mammals suffering from an autoimmune disease characterized in administering to said mammal an effective amount of an agent containing at least one telomeric sequence or fragment of an antibody to said telomeric sequence capable of inhibiting anti-DNA-antibodies present in body fluid of said patient from binding to the patient's DNA, said telomeric sequence being selected from the group consisting of a single-stranded telomeric sequence, a complementary strand thereto, a double-stranded telomere, and a part or a repeat or a combination of any of the foregoing.

43. The therapeutic treatment according to claim 42 wherein said autoimmune disease is selected from the group consisting of Lupus Erythematosus, Rheumatoid Arthritis and Scleroderma.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00117

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 33/564, C07K 1/22, C07K 16/18, A61K 31/70, A61K 39/395
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPI, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proc. Natl. Acad. Sci., Volume 92, March 1995, S. M. Barbas et al, "Human autoantibody recognition of DNA", page 2529 - page 2533, see page 2530, first paragraph, and page 2533 --	1,2,4-12, 9-25,27-35, 37,38
A	Proc. Natl. Acad. Sci., Volume 92, January 1995, Herren Wu et al, "Building zinc fingers by selection: Toward a therapeutic application", page 344 - page 348, figure 1 --	1-3
A	EP 0276984 A2 (DIAGNOSTIC PRODUCTS CORPORATION), 3 August 1988 (03.08.88) --	1-38

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

4 June 1996

Date of mailing of the international search report

12 -06- 1996

Name and mailing address of the ISA/
 Swedish Patent Office
 Box 5055, S-102 42 STOCKHOLM
 Facsimile No. +46 8 666 02 86

Authorized officer

Carl-Olof Gustafsson
 Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00117

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0205643 A1 (RAUTERBERG, E.W., DR.), 30 December 1986 (30.12.86) --	1-38
A	National Library of Medicine, file Medline, NLM accession no. 95031010, Gabibov AG et al: "DNA-hydrolyzing autoantibodies", & Appl Biochem Biotechnol 1994 May-Jun;47(2-3):293-302; discussion 303 --	1
A	J. Am. Chem. Soc., Volume 116, 1994, S. M. Barbas et al, "Recognition of DNA by Synthetic Antibodies" page 2161 - page 2162 --	1
A	Dialog Information Services, Derwent database WPI, Dialog accession no. 009259776, WPI accession no. 92-387189/47, TOSHIBA KK: "Method of detecting gene by reacting sample with antibody to single stranded oligo-nucleotide - of given base sequence, then detecting presence of antigen-antibody reaction", &JP,A,4286957, 921012, 9247 (Basic) --	1
X	WO 9323572 A1 (GERON CORPORATION), 25 November 1993 (25.11.93), page 71, line 20 - line 21; page 75, line 25 - line 28, figure 30 --	18,19,25,26
X	National Library of Medicine, file Medline, NLM accession no. 95058242, Schriever-Schwemmer G et al: "Differentiation of micronuclei in mouse bone marrow cells: a comparison between CREST staining and fluorescent in situ hybridization with centro- meric and telomeric DNA probes", & Mutagenesis 1994 Jul;9(4):333-40 --	18,19,25,26

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00117

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	National Library of Medicine, file Medline, NLM accession no. 95347387, Herrmann M et al: "Preferential recognition of specific DNA motifs by anti-double-stranded DNA autoantibodies", & Eur J Immunol 1995 Jul;25(7):1897-904 -- -----	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00117

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 39, 42, 43
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 39, 42, 43 relates to methods of treatment of the human or animal body by surgery or by therapy diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

CORRECTED

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/96

International application No.

PCT/FI 96/00117

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP-A2-	0276984	03/08/88	AU-A-	1070588	04/08/88
EP-A1-	0205643	30/12/86	NONE		
WO-A1-	9323572	25/11/93	NONE		